Dihydrolipoamide dehydrogenase from halophilic archaeobacteria

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Dihydrolipoamide dehydrogenase has been discovered in the halophilic archaeobacteria for the first time. The enzyme from both classical and alkaliphilic halobacteria has been investigated. (1) The enzyme specifically catalysed the stoichiometric oxidation of dihydrolipoamide by NAD^+ . Enzymic activity was optimal at 2M-NaCl and was remarkably resistant to thermal denaturation. (2) The relative molecular masses (M_r) of the native enzyme from the various species of halobacteria were determined to be within the range 112000–120000. (3) The enzyme exhibited a hyperbolic dependence of catalytic activity on both dihydrolipoamide and NAD^+ concentrations. From these steady-state kinetic measurements the dissociation constant (K_s) of dihydrolipoamide was determined to be $57(\pm 5)\mu\text{M}$. (4) The enzyme was only susceptible to inactivation by iodoacetic acid in the presence of its reducing ligands, dihydrolipoamide or NADH. The rate of inactivation followed a hyperbolic dependence on the concentration of dihydrolipoamide, from which the K_s of this substrate was calculated to be $55(\pm 7)\mu\text{M}$. Together with the steady-state kinetic data, the pattern of inactivations is consistent with the involvement in catalysis of a reversibly reducible disulphide bond, as has been found in dihydrolipoamide dehydrogenase from non-archaeobacterial species. In eubacterial and eukaryotic organisms, dihydrolipoamide dehydrogenase functions in the 2-oxo acid dehydrogenase complexes. These multienzyme systems have not been detected in the archaeobacteria, and, in the context of this apparent absence, the possible function and evolutionary significance of archaeobacterial dihydrolipoamide dehydrogenase are discussed.

In eukaryotes and many aerobic eubacteria, the oxidative decarboxylations of pyruvate and 2-oxoglutarate are carried out by the pyruvate dehydrogenase and the 2-oxoglutarate dehydrogenase multienzyme complexes. Both complexes are multimeric structures [M_r $(3\text{--}10) \times 10^6$] composed of multiple copies of the three constituent enzymes: 2-oxo acid decarboxylase, a lipoate acyl-transferase and dihydrolipoamide dehydrogenase [for reviews, see Reed (1974) and Perham (1975)]. Substrate is carried by lipoic acid residues (Nawa *et al.*, 1960), which are thought to rotate among the catalytic centres of the three component enzymes of each complex (Green & Oda, 1961; Koike *et al.*, 1963; Ambrose & Perham, 1976). The dihydrolipoamide dehydrogenase serves to re-oxidize those

lipoic acid residues reduced in the formation of acyl-CoA, a function which it also fulfils in the branched-chain 2-oxo acid dehydrogenase complexes (Pettit *et al.*, 1978). In all three complexes NAD^+ serves as the electron acceptor.

In anaerobic eubacteria and archaeobacteria, oxidations of pyruvate and 2-oxoglutarate to their corresponding acyl-CoA thioesters are catalysed by less complex oxidoreductases of M_r $(2\text{--}3) \times 10^3$ (Kerscher & Oesterhelt, 1982). In these enzymes NAD^+ does not serve as electron acceptor; rather, electrons are transferred in most cases to ferredoxin, although some organisms utilize flavodoxin (Blaschkowski *et al.*, 1982), and methanogens use the deazaflavin derivative F_{420} (Zeikus *et al.*, 1977). Studies on the ferredoxin oxidoreductases from halophilic and thermoacidophilic archaeobacteria (Kerscher & Oesterhelt, 1981a,b; Kerscher *et al.*, 1982) have demonstrated a four-step catalytic cycle: two one-electron transfers and

Abbreviations used: Dnp-lysine: ϵ -(2,4-dinitrophenyl)-L-lysine; Nbs₂, 5,5'-dithiobis-(2-nitrobenzoic acid) ('DTNB'); GSSG, oxidized glutathione.

a two-step conversion of 2-oxo acid to acyl-CoA by thiamin pyrophosphate acting at a single active site. Lipoic acid is absent from these oxidoreductases, highlighting a fundamental difference between them and the 2-oxo acid dehydrogenase complexes.

All archaeobacterial species so far investigated contain the type of oxidoreductase found in anaerobic eubacteria. Taken with the apparent absence of 2-oxo acid dehydrogenase complexes in these same organisms, Kerscher & Oesterhelt (1982) have suggested that the oxidoreductases evolved long before the dehydrogenase complexes. However, in the present paper we demonstrate the presence of dihydrolipoamide dehydrogenase in halophilic archaeobacteria and find striking similarities with the eubacterial and eukaryotic enzyme that functions in the 2-oxo acid dehydrogenase complexes. The metabolic and evolutionary significance of this discovery is discussed.

Materials and methods

Materials

Chemicals used were of analytical grade or the finest grade commercially available. NAD^+ , NADP^+ , NADH , NADPH , GSSG , citrate synthase (pig heart), fumarase (pig heart), lactate dehydrogenase (pig heart), malate dehydrogenase (pig heart), and 3-phosphoglycerate kinase (yeast) were from Boehringer, Mannheim, Germany; myoglobin (whale skeletal muscle) and Nbs_2 were from Sigma Chemical Co., Poole, Dorset, U.K.; DL-lipoamide, iodoacetic acid and Dnp-lysine were from BDH Chemicals, Poole, Dorset, U.K.; Sephacryl S-200 (superfine grade) was from Pharmacia.

DL-Dihydrolipoamide was prepared by the reduction of DL-lipoamide with NaBH_4 (Reed *et al.*, 1958). The preparation was shown to be 99% pure as judged by titration of the thiol groups of dihydrolipoamide with Nbs_2 .

Bacterial strains and growth

The strains of extreme halophilic bacteria used in this work were kindly provided by Dr. W. D. Grant (Department of Microbiology, University of Leicester, Leicester, U.K.). These were the classical halophiles *Halobacterium halobium* (C.C.M. 2090) and *Halobacterium volcanii* (N.C.M.B. 2012) and the alkaliphilic halophiles *Natronobacterium pharaonis* (N.C.M.B. 2191), *Natronobacterium gregoryi* (N.C.M.B. 2189) and *Natronococcus occultus* (N.C.M.B. 2192) (Collins *et al.*, 1981; Tindall *et al.*, 1983). All strains were grown aerobically in liquid shake culture; *H. halobium* was grown in the medium described by Payne *et al.* (1960), *H. volcanii* in the medium described by Mullakhan-

hai & Larsen (1975) and the alkaliphilic halophiles in the medium described by Tindall *et al.* (1980).

Cell extraction

Cells (0.2 g wet weight) were suspended in 1 ml of 20 mM-Tris/HCl buffer, pH 8.0, containing 2 mM-EDTA, 0.1 M-KCl and 4 M-NaCl. The suspension was sonicated at 0°C for three periods of 30 s at 40 W with a 3 mm probe on an Ultrasonic disintegrator; the cell debris was removed by centrifugation at 10000g (r_{av} 5.5 cm). The supernatant was stored at 4°C.

Enzyme assays

(a) *Dihydrolipoamide dehydrogenase*. Dihydrolipoamide dehydrogenase activity was assayed at 25°C in 50 mM-potassium phosphate, pH 7.0, 1 mM- NAD^+ , 0.4 mM-dihydrolipoamide and 2 M-NaCl. The reaction, in a final volume of 1 ml, was started with enzyme and its progress was monitored by the increase in A_{340} .

(b) *Alcohol dehydrogenase*. Alcohol dehydrogenase was assayed at 25°C in 50 mM-potassium phosphate, pH 7.0, 1 mM- NAD^+ , 35 mM-ethanol and 2 M-NaCl. The reaction, in a final volume of 1 ml, was started with enzyme and its progress monitored by the increase in A_{340} .

(c) *Glutathione reductase*. Glutathione reductase was assayed at 25°C in 50 mM-potassium phosphate (pH 7.0)/0.1 mM- NADPH /0.2 mM- GSSG /2 M-NaCl. The reaction, in a final volume of 1 ml, was started with enzyme and was monitored by the decrease in A_{340} .

Gel filtration

Gel filtration of halobacterial extracts was carried out at 25°C on a column (2 cm $\times$ 33 cm) of Sephacryl S-200 (superfine grade) in 20 mM-Tris/HCl, pH 8.0, containing 1 mM-EDTA and 4 M-NaCl. Blue Dextran and Dnp-lysine were included to measure the exclusion and retention volumes respectively. Standard proteins were run separately and in the absence of NaCl; again, Dextran Blue and Dnp-lysine were included to enable the elution volumes in the absence and presence of 4 M-NaCl to be compared. The standard proteins were pig heart fumarase (M_r 194000), pig heart lactate dehydrogenase (M_r 142000), pig heart citrate synthase (M_r 98000), pig heart malate dehydrogenase (M_r 67000), yeast 3-phosphoglycerate kinase (M_r 47000) and whale skeletal-muscle myoglobin (M_r 17200). For each protein, values of K_d (the distribution coefficient) were calculated as described by Belew *et al.* (1978):

$$K_d = (V_e - V_0)/(V_{re} - V_0)$$

where V_e , V_0 and V_{re} represent the elution volumes

of the protein, Dextran Blue and Dnp-lysine respectively.

Chemical modification with iodoacetic acid

Modification of dihydrolipoamide dehydrogenase with iodoacetic acid was carried out at 25°C in 50 mM-potassium phosphate (pH 7.0)/2 M-NaCl and in the presence and absence of either NADH or dihydrolipoamide. (The concentration of iodoacetic acid and of the substrates were varied according to the experiment and are specified in each case in the text.)

Results

The presence of dihydrolipoamide dehydrogenase activity in halobacteria

Dihydrolipoamide dehydrogenase activity, as defined in the following equation:

was detected in both the classical halophiles *H. halobium* and *H. volcanii* and in the alkaliphilic halophiles *Nb. pharaonis*, *Nb. gregoryi* and *Nc. occultus*. Specific activities in cell-free extracts were in the range of 0.05–0.10 μmol of NADH produced/min per mg. Enzymic activity was assayable in both directions, but in all experiments reported in the present paper the reduction of NAD^+ by dihydrolipoamide was monitored (see the Materials and methods section).

Under the defined assay conditions the rate of production of NADH was directly proportional to the amount of enzyme extract in the assay. All enzymic activity was destroyed on boiling the extract for 15 min. The enzyme was specific for NAD^+ , no oxidation of dihydrolipoamide being detectable when NADP^+ was substituted for NAD^+ . Moreover, no endogenous reduction of NAD^+ by the extracts was observable in the absence of dihydrolipoamide. Since the dihydrolipoamide is added to the assay mixture as a solution in ethanol, all extracts were assayed for the presence of alcohol dehydrogenase. No activity was detected, confirming that it is the dihydrolipoamide that reduces the NAD^+ . In support of this, the stoichiometry of the reaction was measured by monitoring NADH production at 340 nm and by titrating the unchanged dihydrolipoamide with Nbs_2 . It was found that with each enzyme extract 1 mol of NADH was produced/mol of dihydrolipoamide oxidized.

The possibility was considered that the observed dihydrolipoamide dehydrogenase activity is a side reaction of another oxidoreductase enzyme. Perhaps the most likely candidate is glutathione

reductase (EC 1.6.4.2), which is closely related to eubacterial and eukaryotic dihydrolipoamide dehydrogenase in catalytic mechanism and the amino acid sequence around the catalytically active disulphide bridge (Williams, 1976; Perham *et al.*, 1978). Halobacterial extracts were found to contain glutathione reductase activity, but at a level less than 20% of that of the dihydrolipoamide dehydrogenase. Furthermore, these two enzymic activities were separable by gel filtration, demonstrating that they reside on different proteins.

Kinetic properties of dihydrolipoamide dehydrogenase

The enzymes from both classical and alkaliphilic halophiles were assayed in 0–4 M-NaCl. In common with other halophilic enzymes, dihydrolipoamide dehydrogenase activity increased markedly as the salt concentration was raised from 0 to 1 M-NaCl and reached a maximum at 2 M-NaCl.

The enzyme from each extract showed a hyperbolic dependence of rate on each substrate. The dependence of *Nb. gregoryi* dihydrolipoamide dehydrogenase activity on the concentration of dihydrolipoamide is shown in Fig. 1. The data were analysed by the direct linear plot (Eisenthal & Cornish-Bowden, 1974) and are graphically presented in the 'half-reciprocal' plot. The dissociation constant (K_s) for dihydrolipoamide, given by the point of intersection in the half-reciprocal plot shown in Fig. 1 (Wharton & Eisenthal, 1981), was determined to be 57 (± 5) μM . Further analysis of these data gave K_m values for dihydrolipoamide of 17 (± 2) μM and for NAD^+ of 1.1 (± 0.2) mM. Similar kinetic parameters for dihydrolipoamide dehydrogenase were found in extracts of the other halobacteria.

Thermal stability

Halophilic dihydrolipoamide dehydrogenases were found to be remarkably resistant to thermal denaturation. Incubation of the enzyme at 95°C for 15 min in the presence of 4 M-NaCl resulted in no detectable loss of enzyme activity, although, as stated previously, boiling the enzyme did cause inactivation. Under identical buffer conditions other halophilic enzymes (e.g. citrate synthase) were completely inactivated at 60°C in 10 min.

Determination of the relative molecular masses of the native enzymes

Relative molecular masses (M_r) were determined by zonal gel filtration on Sephacryl S-200 by the method of Andrews (1965) as described in Materials and methods section. In order to compare the elution volumes of the standard proteins, necessarily run in the absence of high concentrations of NaCl, with those of halo-

Fig. 1. Dependence of dihydrolipoamide dehydrogenase activity on dihydrolipoamide concentration. Dihydrolipoamide dehydrogenase from the alkaliphilic halophile *Nb. gregoryi* was assayed spectrophotometrically at 340 nm as described in the Materials and methods section. The dependence of enzymic activity on the concentration of dihydrolipoamide was determined at NAD^+ concentrations of 0.25 mM (●), 0.5 mM (○) and 1 mM (▲). The data were analysed by the direct linear plot and are plotted in the half-reciprocal form of dihydrolipoamide concentration/enzymic activity ($[S]/v$) versus dihydrolipoamide concentration. Enzyme activity is in arbitrary units.

Fig. 2. Gel filtration on Sephacryl S-200: estimation of relative molecular masses of dihydrolipoamide dehydrogenases.

Gel filtration on Sephacryl S-200 (superfine grade) was performed as described in the text. K_d is the distribution coefficient, calculated as described in the Materials and methods section. Standard proteins: A, pig heart fumarase; B, pig heart lactate dehydrogenase; C, pig heart citrate synthase; D, pig heart malate dehydrogenase; E, yeast 3-phosphoglycerate kinase; F, whale skeletal-muscle myoglobin. The bars represent the s.e.m. of the K_d values of each protein determined in five separate filtration experiments. G represents the range of K_d values determined for dihydrolipoamide dehydrogenase from *H. halobium*, *H. volcanii*, *Nb. pharaonis*, *Nb. gregoryi* and *Nc. occultus*.

bacterial dihydrolipoamide dehydrogenase determined in the presence of 4M-NaCl, the data from the gel filtrations were plotted in the form $\log M_r$ versus K_d (Fig. 2). A good fit to a straight line is observed for the standard proteins. With each halophilic extract, dihydrolipoamide dehydrogenase was eluted as a single peak. Values of K_d for the enzyme from the various organisms were all in the range 0.173–0.181, giving the following individual values of M_r : *H. halobium*, 112000; *H. volcanii*, 119000; *Nb. pharaonis*, 112000; *Nb. gregoryi*, 119000; and *Nc. occultus*, 120000. In no case was any dihydrolipoamide dehydrogenase activity found in the void volume of the elution profile where it might have been expected if present as a component of a 2-oxo acid dehydrogenase complex.

Chemical modification of dihydrolipoamide dehydrogenase from *Nb. gregoryi*

Dihydrolipoamide dehydrogenase from eubacterial and eukaryotic species functions by a catalytic mechanism involving alternate oxidation and reduction of an intrachain disulphide bridge [reviewed by Williams (1976)]. Thus, in the presence of either dihydrolipoamide or NADH, the enzyme exists in the dithiol form and should be susceptible to inactivation by thiol reagents such as iodoacetic acid. In the absence of these ligands, no

Fig. 3. Chemical modification of dihydrolipoamide dehydrogenase with iodoacetic acid

Cell-free extracts of *Nb. gregoryi* were incubated at 25°C in 50 mM-potassium phosphate (pH 7.0)/2 M-NaCl in the presence of (a) 18 mM-iodoacetic acid and 0.3 mM-NADH (▲); (b) 18 mM-iodoacetic acid and 0.15 mM-dihydrolipoamide (●); (c) 18 mM-iodoacetic acid (□); (d) 0.3 mM-NADH (△) and (e) 0.15 mM-dihydrolipoamide (○). Dihydrolipoamide dehydrogenase was assayed spectrophotometrically at 340 nm as described in the Materials and methods section.

Fig. 4. Chemical modification of dihydrolipoamide dehydrogenase with iodoacetic acid: dependence of rates of inactivation on the concentration of iodoacetic acid. Pseudo-first-order rate constants for the inactivation of *Nb. gregoryi* dihydrolipoamide dehydrogenase by iodoacetic acid in the presence of 0.3 mM-NADH (▲) and of 0.15 mM-dihydrolipoamide (●) were calculated from semilogarithmic plots such as those given in Fig. 3.

active-site modification can take place. This phenomenon, whereby substrate and product render active-site residues susceptible to chemical modification rather than protect them, is most unusual and characteristic of dihydrolipoamide dehydrogenase. Therefore the effects of thiol-group modification on the halophilic enzymes were examined.

Dihydrolipoamide dehydrogenase from both classical and alkaliphilic halophiles was inactivated by iodoacetic acid; the kinetics of inactivation were consistent with the enzyme possessing a catalytic mechanism similar to that operating in eubacterial and eukaryotic organisms. The analysis of the modification of the *Nb. gregoryi* enzyme is presented below.

Dihydrolipoamide dehydrogenase from *Nb. gregoryi* was inactivated by iodoacetic acid in the presence of NADH or dihydrolipoamide (Fig. 3), but no loss of activity was observed in the presence of either iodoacetic acid or substrates alone. This ligand-induced sensitization of an enzyme to inactivation by a chemical reagent is similar to the phenomenon termed 'syncatalytic modification' (Christen & Riordan, 1970), i.e. modification synchronous with the catalytic process. The inactivations appeared to be pseudo-first-order reactions and the rates of loss of enzyme activity were directly proportional to the concentration of iodoacetic acid (Fig. 4). If indeed the inactivation is

only possible through the binding of substrate to the enzyme with subsequent reduction of an active-site disulphide bond, then the rate of inactivation at any fixed concentration of iodoacetic acid should be determined by the degree of saturation of the enzyme with its ligand. Assuming the following mechanism from the above observations:

then it can be shown that:

$$k_{\text{obs.}} = \frac{k'[\text{L}]}{K + [\text{L}]} \quad (1)$$

where $k_{\text{obs.}}$ is the observed pseudo-first-order rate constant of inactivation of the enzyme by iodoacetic acid.

Fig. 5. Chemical modification of dihydrolipoamide dehydrogenase with iodoacetic acid: dependence of rate of inactivation on the concentration of dihydrolipoamide

Cell-free extracts of *Nb. gregoryi* were incubated at 25°C in 50 mM-potassium phosphate (pH 7.0)/2 M-NaCl in the presence of 18 mM-iodoacetic acid and various concentrations of dihydrolipoamide. Pseudo-first-order rate constants for the inactivation ($k_{\text{obs.}}$) were calculated for each concentration of dihydrolipoamide (L). The data are plotted as $[L]/k_{\text{obs.}}$ versus $[L]$, after analysis by a weighted fit to the direct linear plot.

tic acid at concentration $[R]$ in the presence of ligand at concentration $[L]$ and $k' = k[R]$ where k is the second-order rate constant for the reaction of (EL) with iodoacetic acid. Provided that k' is much less than the dissociation rate constant of EL, K is approximately equal to the dissociation constant of EL. In the present case k' is smaller, by a factor of more than 10^3 , than the lowest values normally found for the dissociation rate constants of enzyme complexes with small ligands (see, e.g., Fersht, 1977). Thus K is a reasonably accurate estimate of the dissociation constant, K_s .

The dihydrolipoamide dehydrogenase from *Nb. gregoryi* was modified with 18 mM-iodoacetic acid in various concentrations of dihydrolipoamide. Values of $k_{\text{obs.}}$ were calculated from semilogarithmic plots of enzymic activity versus time. The dependence of $k_{\text{obs.}}$ on the concentration of dihydrolipoamide was then analysed by the direct linear plot (Eisenthal & Cornish-Bowden, 1974) and the data are plotted in the 'half-reciprocal' form of eqn. (1), i.e. $[L]/k_{\text{obs.}}$ versus $[L]$ (Fig. 5). The values of $k_{\text{obs.}}$ and $[L]$ give a good fit to eqn. (1). The intercept on the abscissa of Fig. 5 represents a K_s for dihydrolipoamide of $55(\pm 7) \mu\text{M}$. This value is in good agreement with that obtained from the kinetic studies and so provides strong evidence

that the exposure of essential groups on the enzyme by dihydrolipoamide is a function of the substrate's participation in the catalytic process. The value of k , the second-order rate constant for the chemical inactivation, was found to be $7.6(\pm 0.3) \text{M}^{-1} \cdot \text{min}^{-1}$.

It should be noted that, at pH 7.0, iodoacetic acid does not react with the thiol groups of dihydrolipoamide (M.J. Danson, unpublished work). This observation was confirmed in the present experiments by titration of the dihydrolipoamide with Nbs_2 .

Discussion

The results in the present paper represent the first report of the presence of dihydrolipoamide dehydrogenase in the halobacteria. Their significance stems from previous studies on the oxidation of 2-oxo acids such as pyruvate and 2-oxoglutarate in the archaeobacteria, of which halobacteria are members. In all archaeobacteria tested so far, these oxidative decarboxylations are catalysed by ferredoxin oxidoreductases (Kerscher & Oesterhelt, 1981a,b, 1982; Kerscher *et al.*, 1982) and, in contrast with the situation in aerobic eubacterial and eukaryotic species, the NAD^+ -linked 2-oxo

acid dehydrogenase complexes have never been detected (Aitken & Brown, 1969; Kerscher & Oesterhelt, 1982; S. Hall & M. J. Danson, unpublished work). Taken with the presence of the oxidoreductases in anaerobic eubacteria, Kerscher & Oesterhelt (1982) suggest that these ferredoxin-linked enzymes already existed before the divergence of the three kingdoms, Archaeobacteria, Eubacteria and Eukaryotes, and that the emergence of the 2-oxo acid dehydrogenase complexes was a later evolutionary process after the development of oxidative phosphorylation.

The discovery of dihydrolipoamide dehydrogenase activity in the extreme halophiles may require a modification to this evolutionary scheme. In eubacteria and eukaryotes the only known function of this enzyme is the reoxidation of dihydrolipoamide formed in the catalytic reaction of the 2-oxo acid dehydrogenase complexes (Reed, 1974; Perham, 1975; Pettit *et al.*, 1978). Thus it might not be unreasonable to suppose that dihydrolipoamide dehydrogenase fulfils a similar role in the halobacteria. This view is supported by the close similarity of the enzyme to its counterpart in non-archaeobacterial species. Thus we have shown that the halophilic enzyme is catalytically specific for dihydrolipoamide and NAD^+ , has an M_r of 112000–120000 and is extremely thermostable. [The enzyme from both eubacterial and eukaryotic organisms also possesses this specificity, is a dimeric protein of M_r 113000 and is resistant to a number of denaturing conditions, including high temperatures, urea and proteolytic enzymes (Williams, 1976).] Additionally, in a reaction unique to dihydrolipoamide dehydrogenases, the halophilic enzyme becomes susceptible to inactivation by iodoacetic acid only in the presence of its reducing ligands, dihydrolipoamide or NADH. This process, whereby substrate permits the modification of essential thiol groups, is entirely consistent with the catalytic mechanism operative in other dihydrolipoamide dehydrogenases, namely the alternate oxidation and reduction of an active-site disulphide bond (Williams, 1976).

A more detailed analysis of the halobacterial enzyme must await its purification. However, the features that it has in common with non-archaeobacterial dihydrolipoamide dehydrogenases may reflect the presence of functional pyruvate and 2-oxoglutarate dehydrogenase complexes in the halobacteria. In other species these multienzyme systems contain numerous subunits which, even in extreme halophiles, might be dissociated by extraction and dilution into very high salt concentrations. This would cause the loss of overall complex activities without the necessary inactivation of each component enzyme. The pres-

ence within one organism of both NAD^{+} - and ferredoxin-linked enzymes for the oxidative decarboxylation of 2-oxo acids is not without precedent; it has been shown, for example, that *Escherichia coli* possesses low levels of pyruvate ferredoxin oxidoreductase in addition to higher catalytic activities of the pyruvate dehydrogenase multienzyme complex (Blaschkowski *et al.*, 1982).

It is of course possible that the dihydrolipoamide dehydrogenase may serve an, as yet, unrecognized role in the halobacteria, and that the failure to detect oxo acid dehydrogenase activity does indeed reflect their true absence from these archaeobacteria. Furthermore, until the metabolic function of the dehydrogenase is better defined, there remains the inevitable uncertainty that another physiological substance may be a better substrate for the enzyme than dihydrolipoamide. However, the observed similarities to other dihydrolipoamide dehydrogenases would argue against this being the case.

Whatever its role in halobacteria it is clear that both metabolic and evolutionary considerations warrant the purification and a more detailed kinetic and molecular characterization of this dihydrolipoamide dehydrogenase. In parallel with this it may be prudent to make further investigations into the presence or absence of the 2-oxo acid dehydrogenase complexes in these organisms.

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